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Isolation and characterization of *gpd* gene from selective lignin degrading fungus, *Ceriporiopsis subvermispora*

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White rot fungi are known to degrade plant cell wall lignin and also various aromatic pollutants. Intensive research has been done to make use of their special function for a novel carbon recycle system and also bioremediation of polluted environments, which contribute for development of a sustainable society. Especially, a selective white rot basidiomycete, *Ceriporiopsis subvermispora* is a promising microorganism effective as a biocatalyst to degrade lignin in industrial processes, including pulp and paper manufacture, and conversion of lignocellulosic biomass to various compounds. However, there is no report on development of DNA-mediated transformation system in this fungus. Transformation techniques are valuable tools for not only molecular biological analysis of the selective lignin degrading system but also strain improvement of desired catalytic phenotype.

Glyceraldehyde-3-phosphate dehydrogenase (GPD) is a key enzyme in glycolysis and gluconeogenesis. It is highly and constitutively expressed and, in higher eukaryotes, GPD protein comprises up to 5% of the soluble cellular protein. From this property the *gpd* promoter has been used to drive recombinant genes in various microorganisms. In this study, I attempted to obtain a novel transformation vector using *gpd* promoter cloned from *C. subvermispora*.

C. subvermispora strain ATCC 90466 were used throughout this work. Genomic DNA was extracted from mycelium cultured on YMPG liquid medium (0.2% Yeast extract, 1% Malt extract, 0.2% Bactotryptone, 1% glucose 0.1% L-aspargine and 0.1% $MgSO_4$), using the CTAB method. DNA fragments covering 1.3-kb promoter and terminator sequences were amplified using TaKaRa LA PCR *in vitro* cloning kit. Nucleotide sequence of the cloned fragments were determined and characterized, suggesting that *C. subvermispora gpd* gene encodes a 337-aa protein. Presence of six introns was predicted and the deduced amino acid sequence of *C. subvermispora gpd* gene showed high similarity to those of other basidiomycetes. Actual transcription from *gpd* was confirmed using RT-PCR technique and the expression was controlled by glucose in the medium.

Transforming vector plasmids were designed to contain the promoter and terminator sequences from *gpd* combined to a hetrologous drug resistant marker gene, hygromycin B phosophotransferase (*hph*). Two recombinant plasmids, with or without the first intron , were constructed, since Logones *et al.* reported that introns enhanced expression of *ABH1* in *Schizophillum commune* [1].

REFERENCES

[1] Lugones, L.G., Dcholtmeijer K., Klootwijk R. and Wessels J.G.H. (1999) Mol. Microbiol. 32: 681-689.